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A study on pollination ecology of butterfly pea, *Clitoria ternatea* L. (Fabaceae)

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ABSTRACT

Clitoria ternatea is a herbaceous perennial climbing herb and grows as an aggressive colonizer in several habitats with poor to moderate soil nutrient environment. It has blue- and white-flowered varieties with similar floral structural and functional characters. The flowers are chasmogamous, nectariferous, resupinate and hermaphroditic. They are typically papilionaceous with brush pollination mechanism, weak protandry and resort to autonomous autogamy and facultative xenogamy to maximize fruit and seed set rates in open-pollinations. Seed dispersal modes include autochory and hydrochory. This plant is widely valued in Indian Medicine System for treating various human diseases and ailments, as forage and fodder crop, natural food colorant, antioxidant, ornamental plant and as a re-vegetation species in the coal mine sites. Therefore, *C. ternatea* can be commercially and ecologically exploited.

Keywords: *Clitoria ternatea*, resupinate flowers, brush mechanism, facultative xenogamy, bee-pollination, autochory, hydrochory.

1. INTRODUCTION

In Papilionoid flowers, the corolla is pentamerous consisting of a standard petal, two wing petals and two keel petals. The keel petals conceal the stamens and stigma within blocking the free access to pollen by flower visitors (Westerkamp 1997). In most species, the standard petal is placed in the upper position and the keel and wing petals take the lower position and act as the landing platform for insect visitors and pollen is deposited onto the ventral side of the probing insects. In some species, the flowers are resupinate in which the keel and wing petals are placed in the upper position while the standard petal is placed in the lower position, which acts as the landing place for insect visitors and the pollen is deposited onto the dorsal side of probing insects (Westerkamp and Claβen-Bockhoff 2007; Amaral-Neto et al. 2015). The flower mechanism of non-resupinate and resupinate flowers demands specific positioning and specific movements by bee visitors and an integration of their body parts to work simultaneously. Small bees are not capable to access nectar hidden at the base of keel petals because they do not have the strength to operate the flower mechanism. Medium to large bees are capable to access nectar placed at the base of keel petals as they have necessary strength to operate the flower mechanism (Westerkamp 1997; Cordoba and Cocucci 2011).

The genus *Clitoria* belongs to Fabaceae family, Phaseoleae tribe and Clitoriinae sub-tribe (NPGS 2008). *Clitoria* is a Latin name for the fanciful resemblance of the flower to human female sexual apparatus, keel formed by two petals resembling the clitoris escorted by wing petals resembling the labia minor and the standard petal resembling the labia major (Frantz 2000). This genus comprises sixty species distributed mostly in the tropical belt with a few species occurring in temperate areas (Frantz 1990). It is characterized by its woody nature, showy, resupinate papilionaceous flowers with an infundibular calyx enclosed by persistent bracteoles, persistent stipules and stipels, and stalked ovaries with a geniculate, bearded style (Frantz 1990). Further, it is distinguished by the presence of geniculate styles and the absence of both the canavanine in seed and appendages on the standard (Lavine and Alfonso 1990). *Clitoria* species produce chasmogamous flowers adapted for insect pollination and cleistogamous flowers adapted for self-pollination. Flower color, position and structure varies with each species of this genus (Gomez and Kalamani 2003). *C. fragrans* is a rare perennial endemic herb. It has mixed mating system; the chasmogamous flowers are open and insect pollinated while cleistogamous flowers are closed and self-pollinated (Lewis 2007).

In *Clitoria* genus, *C. ternatea* is widely distributed throughout Africa, Asia, Australia, North and South America, and Northwestern, South Central and Southwestern Pacific (Al-Snafi 2016). It has two flower varieties, blue and white and both varieties are widespread in tropical areas and occur in the same habitats (Prafulkumar 2011). This species is widely used in Indian Medicine System. The roots are used to treat severe bronchitis, asthma, hectic fever, swelling, pain and bleeding piles. Leaf juice is used as nasal drops for relief from headache (Kirtikar and Basu 1991). In Ayurvedic medicine, the roots, seeds and leaves are used as a brain tonic to promote memory and intelligence (Mukherjee et al. 2008). The plant has long been cultivated as a forage and fodder crop and has potential applications both in modern medicine and agriculture, and as a source of natural food colorants and antioxidants (Reid and Sinclair 1980; Barrow and Ribeiro 1983; Hall 1985). The flowers are used to color food in Southeast Asia. An aqueous extract is used to color glutinous rice in Malay cooking (Jain et al. 2003). The plant is a highly palatable forage legume which is preferred by livestock over other leguminous plants. It re-grows excellently within a short duration after grazing and also produces higher yields. It is cultivated as a green manure, ground cover crop, for rotational grazing, protein bank, hay and silage production (Gomez and Kalamani 2003; Cook et al. 2005). The pods are edible when tender. It is cultivated as an ornamental plant as its cultivation requires little care (Prafulkumar 2011).

The available information on certain aspects of reproductive ecology of *C. ternatea* is reviewed. Staples (1992) reported that *C. ternatea* flowers are cleistogamous, most flowers are self-pollinated but out-crossing also occurs to a small extent. The flowers are visited by insects, primarily bees. Cook et al. (2005) and Chen et al. (2018) reported that *C. ternatea* is a self-pollinated plant. Girish Kumar (2017) reported that *C. ternatea* is pollinated by bees, moths, butterflies; it exhibits vivipary during non-seasonal rains. Prafulkumar (2011) reported that *C. ternatea* produces blue- and white-flowered varieties in which piston mechanism is functional for the occurrence of pollination; it is a facultative autogamous and insect pollinators are not compulsory for effective pollination, and wind facilitates self-pollination. The same author also mentioned that this species is either facultative xenogamous or has a system having an indication of different pollination behavior. It is visited by bees, ants, beetles, flies, butterflies and birds. These reports are contradictory with reference to sexual system and pollination system and hence warrant for detailed study. The need to study the reproductive ecology of *C. ternatea* is further substantiated by its use for various purposes in traditional and modern medicine, and as food colorant. Therefore, the present study is an attempt to provide the floral biology, pollination mechanism, pollinators, fruiting ecology and seed dispersal in *C. ternatea*.

2. MATERIALS AND METHODS

Clitoria ternatea growing in wild patches of Andhra University campus was used for study during June 2019 to December 2020. It grows along with seasonal herbaceous flora thrive well in wet season. This species was investigated for plant phenology, flowering season, flower morphology, floral biology, foraging activity, pollination mechanism, pollinators, fruiting and seed dispersal aspects. Since this species represented blue- and white-flowered varieties, all these aspects were accurately observed to record between varieties. Stigma receptivity was tested using H_2O_2 test as prescribed in Dafni et al. (2005). A total of 75 flowers were tagged on different plants and followed for six weeks to record fruit development period and fruit and seed set rates. *In situ* observations were made on fruit dehiscence and seed dispersal and seed germination aspects to record the modes of seed dispersal and germination ability depending on soil moisture status.

3. RESULTS

Phenology and flowering: It is a perennial herbaceous climbing herb with deep root system. It grows well in a variety of soil types from sandy to heavy clay soils with low to moderate fertility. It occupies disturbed and open habitats as an aggressive colonizer

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with ability to displace or outcompete native flora. The stem is twining and sparsely pubescent and slightly erect at the base. Leaves are imparipinnate with 5 petiolate elliptic leaflets. It grows throughout the year but prolific growth and flowering occurs during August-November, the period when the soil has enough moisture due to wet season. The plant has two varieties, blue or indigo- and white-flowered varieties (Figure 1a,b). Both varieties are common and occupy various habitats and grow together as well.

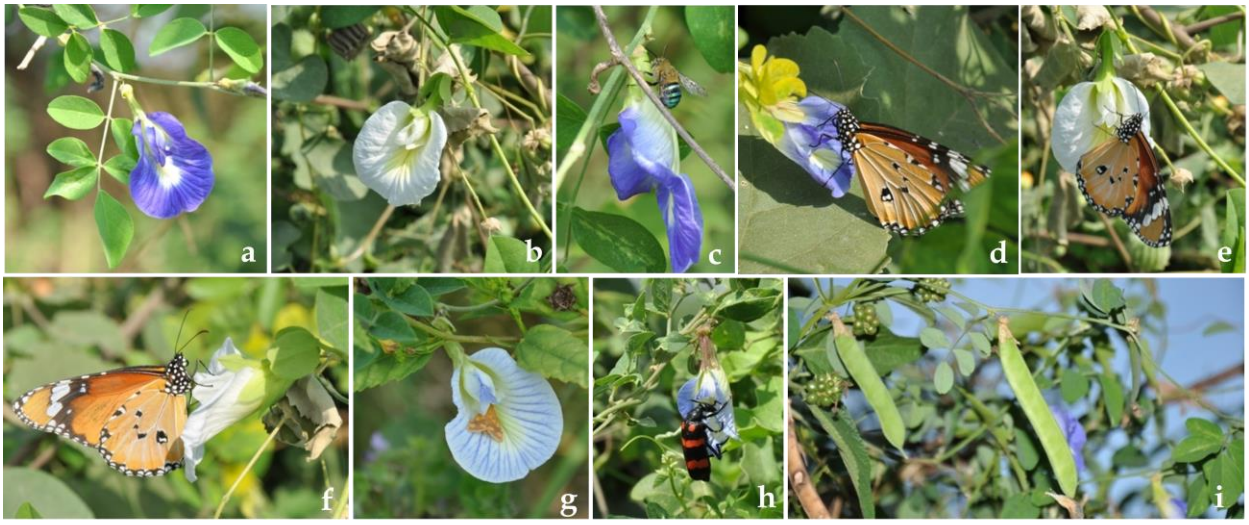

Figure 1. *Clitoria ternata:* a. Twig with blue flower variety, b. White flower variety, c. *Anthophora* sp. making perforation to rob nectar, d-f. Nymphalid butterfly, *Danaus chrysippus* probing for nectar, g. Unidentified moth probing for nectar from white flower variety, h. *Mylabris phalerata* feeding on flower, i. Fruits (pods).

Flower morphology and biology: In both blue and white varieties, the flowers are solitary, pedicellate and born in axillary position. They are large, papilionaceous, 42 mm long, 32 mm wide, zygomorphic and bisexual. Floral morphological and functional characters are almost similar in both varieties. Mature buds open during 0700-0800 h during wet season and during 0600-0800 h during winter and summer season. The calyx has five green sepals fused at base and free towards apex. The corolla is resupinate with one standard petal placed below and two wing petals and two keel petals placed above. In blue-flowered variety, wing and keel petals are completely blue while the standard petal is blue with white center and up to the base. In white-flowered variety, wing and keel petals are white while the standard petal is white with yellow center and up to the base. Further, a few individuals of white-flowered variety were found to produce white flowers with bluish tinge. In both blue and white-flowered varieties, the standard petal is 41 mm long, wing petals 26 mm long and keel petals 19 mm long. The keel petals conceal the stamens and stigma. The stamens are ten, 21 to 23 mm long, diadelphous with nine fused and 1 free arrangement. Anthers are basifixed, 2 mm long, dithecous and introrse. Pistil is 25 mm long and consists of unicarpellary, unilocular ovary with ten ovules arranged on marginal placentation, 17 mm long simple style and incurved stigma; the terminal portion of the style and the stigma have a brush of hairs and extend beyond the height of the stamens.

In open flowers of both varieties, only the standard petal unfolds completely while the wing petals are partly open. The keel petals do not unfold and hold the stamens and stigma inside. Anther dehiscence occurs in mature buds by longitudinal slits. The pollen grains are monads, prolate, tricolporate, psilate, 42.70 x 21.11 μm, elliptical on equatorial outline and triangular-convex on polar outline. The stigma is receptive after anthesis and extends until the noon of the following day. Nectar is secreted during mature bud around the base of the ovary which is enclosed by staminal column which in turn is covered by the corolla base.

Foraging activity and pollination: In both varieties of this species, the standard petal was used as landing place by insect pollinators for probing the flowers to collect the forage. The insect foragers visiting the flowers day long were bees, butterflies, moths and beetles; the same species visited both varieties without any discrimination. The bees were *Apis dorsata, Anthophora* sp. and *Xylocopa latipes*; these bees were consistent and regular foragers but the last bee species was represented by juveniles only. These bees foraged from the flower front by landing on the standard petal in a legitimate way. In this legitimate flower-probing behavior, the stigma protrudes from the keel tip, comes into contact first with the probing bee, receives external pollen if already present on the visiting bee and the style brushes the pollen on the dorsal side of the bee, all these events occur in quick succession to result in cross- or self-pollination. The style and stigma return back to their original position inside the keel upon the release of

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pressure by the departing bee. The bees made repeated visits to the same flowers in search of nectar promoting pollination rate by depositing more pollen on the stigma facilitating the fertilization of many or all ovules of the ovary. They rarely made attempts to collect pollen from the keel by turning their bodies upside down and such a foraging behavior was also treated to be effecting pollination. *Anthophora* bees additionally adopted illegitimate foraging behavior to collect nectar by making puncture/hole at the base of corolla and/or calyx tube (Figure 1c). This illegitimate nectar collection resulted in the depletion of nectar without pollination since the sexual apparatus part of the flower was bypassed. The butterflies were the Papilionid, *Papilio demoleus* and the Nymphalid, *Danaus chrysippus* (Figure 1d-f); they were regular foragers throughout the day and probed the flowers legitimately from the flower front by inserting their proboscis through wing and keel petals but not by depressing the standard petal and in this process, the contact between the proboscis of the probing butterfly and the stigma/style to result in either cross- or self-pollination was not definite. The moth was unidentified but it was very occasional forager for nectar and its role to trip the keel to effect pollination and access nectar was ruled out as it was a very light weight small moth with a short proboscis (Figure 1g). *M. phalerata* was flower-feeder and fed on all petals of corolla along with sex organs concealed in the keel; its flower-feeding behavior was treated to be negatively affecting the reproductive success of the plant (Figure 1h).

Fruiting and seed dispersal: In both varieties, fruit initiation occurs following pollination and fertilization. Fruits mature within 4-5 weeks (Figure 1i). In open pollinations, fruit set is 78% while seed set is 73%. Fruit is a pod, linear, flattened, 50-70 mm long, 8-13 mm wide and bears 5-10 seeds. The seeds are brown to black, 4-6 mm long, 3-4 mm wide, slightly flattened, shiny and minutely pitted. Mature pods dehisce when fully dry to disperse seeds which subsequently reach the ground and germinate immediately if soil is sufficiently wet or else remain in the soil and germinate when soil is enriched with moisture.

4. DISCUSSION

Clitoria ternatea grows year-long as an aggressive colonizer in a variety of habitats ranging from sandy soils to heavy clay soils with poor to moderate nutrient content and displaces or outcompetes with the simultaneously growing species. Newstrom et al. (1994) reported that flowering plants display four basic flowering patterns, continual, sub-annual, annual and supra-annual. These flowering patterns regulated by moisture availability, rainfall seasonality, inter-specific competition and pollinator availability. In *C. ternatea*, the flowering is continual throughout the year in areas where soil is consistently wet and seasonal in areas where soil is seasonally wet. The plant produces blue-flowered and white-flowered varieties and share the same habitats and reproduce well using the same pollinator fauna. Certain individuals of *C. ternatea* in this study produced white flowers with bluish tinge which is very distinct on the standard petal indicating that white flower variety probably evolved from blue flower variety in order to attract pollinator insects in certain areas where blue corolla is disadvantageous to attract intended pollinator insects.

Gomex and Kalamani (2003) reported that *Clitoria* species produce chasmogamous entomophilous flowers and cleistogamous self-pollinated flowers (Gomez and Kalamani 2003). Lewis (2007) reported that *C. fragrans* produces chasmogamous flowers intended for insect-pollination and cleistogamous flowers intended for self-pollination. Staples (1992) reported that *C. ternatea* flowers are cleistogamous, most flowers are self-pollinated but out-crossing also occurs to a small extent due to pollination by insects, primarily bees. In this study, it is found that blue and white-flowered varieties of *C. ternatea* produce only chasmogamous nectariferous flowers which are open for insect pollination.

Westerkamp (1996) and Galloni et al. (2007) reported that the typical flower in Fabaceae is hermaphroditic and strongly zygomorphic adapted to either self- or cross-pollination. Four pollination mechanisms have been classified in this family - explosive, valvular, piston and brush type. In the explosive mechanism, the staminal column within the keel is held under tension and upon the release of tension, this staminal column snaps forward against the standard petal to release all the pollen instantly; the staminal column does not return back into the keel once it is released. In the valvular mechanism, the upper rim of the keel is unsealed, opens along its total length when the keel is pushed downwards by the visiting insect and closes back when the insect leaves the flower; pollen can be released repeatedly to several visiting insects. In the piston mechanism, the keel tip moves under the pressure of the insect while the sex organs keep their place; pollen is then released from the anthers and pushed out through a hole in the keel tip; pollen can be dispersed with repeated visits. In the brush mechanism, the pistil is longer than the stamens and extends beyond the anthers, and avoids self-pollination; the upper part of the style consisting of erect trichomes acts as a pollen brush. When sufficient pressure is exerted on the standard and wing petals by the visiting insect, the stigma protrudes from the keel tip during which the stigma comes first into contact with the insect receiving external pollen and the style brushes the pollen on the visitor. When pressure is released, style and stigma return to their previous position inside the keel. Frantz (1977) reported that *Clitoria* genus is distinguished from other members of the same sub-tribe by a pollen brush with introrse arrangement of stamens;

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this pollen brush is referred to as bearded or pubescent style by Lavine and Alfonso (1990). Prafulkumar (2011) reported that C. *ternatea* flower has piston mechanism adapted for insect pollination. In this study, it is found that both blue- and white-flowered varieties of *C. ternatea* exhibit brush mechanism as it displays the characters specified by Westerkamp (1996) and Galloni et al. (2007). Further, the terminal part of the style and the stigma possess brush-like hairs enabling them to brush the pollen on the probing insect and the sex organs return back to their original place upon departure of the insect.

Rodri et al. (1999) reported that diadelphous androecium in Fabaceae is linked to the presence of an intra-staminal nectary. Many members of this family secrete nectar in the innermost parts of the base of the staminal filaments and accumulates in the space created between the stamens and the pistil (Bonnier 1879; Muller 1883). In this study, it is found that *C. ternatea* varieties display diadelphous staminal column and produce nectar at the inside base of the staminal column in the space between stamens and pistil. Since *C. ternatea* flowers are resupinate, diadelphous condition enables the probing insect to access nectar with great ease.

Prafulkumar (2011) reported that *C. ternatea* flowers self-pollinate and it is facilitated by wind. In this study, *C. ternatea* varieties, self- and cross-pollination occurs during probing by the bees as they have necessary strength to operate brush pollination mechanism. Further, the loss of turgor pressure due to loss of withdrawal of water from the cells of stigma towards evening of the day of anthesis or on the following day causes the incurved stigma to bend further downwards enabling it to capture the pollen present on the brush part of the style to be pollinated, but this autonomous pollination is subject to the availability of pollen on the hairy brush part of the style.

Prafulkumar (2011) reported that *C. ternatea* is facultative autogamous and insect pollinators are not compulsory for effective pollination. He also mentioned that this species is either facultative xenogamous or has a system having an indication of different pollination behavior. In this study, it is found that *C. ternatea* is facultative xenogamous because the stigma is placed beyond the height of stamens and the hairy brush part of the style and stigma contacts the probing insect first followed by anthers. The floral sex organs return back to the keel to enable the stigma and style to come out and brush the dorsal side of the insect in successive visits to the same flowers to maximize cross-pollination while keeping the option open for self-pollination. Further, the fruit and seed set rate recorded in this study also acknowledge that facultative xenogamy is functional in *C. ternatea*. This study disagrees with the report by Prafulkumar (2011) that *C. ternatea* produces 100% fruit set in spontaneous autogamy, manual selfing, geitonogamy, xenogamy and open-pollination. Further, Prafulkumar (2011) also mentioned that the number of seeds per pod varies from 9.5 in spontaneous autogamy to 7 in open-pollination in *C. ternatea*. But, this study observes that *C. ternatea* produces maximum number of seeds per pod in open-pollination while minimum to moderate number of seeds per pod in other modes of pollination.

Prafulkumar (2011) reported that *C. ternatea* is visited by *Apis dorsata, A. mellifera* (bees), *Lasius niger* (ant), *Coccinella septempunctata* (beetle), *Heiroglyphus banian* (grasshopper), *Papilio demoleus* (Papilionid butterfly), *Musca domestica* (fly) and *Passer domesticus* (House Sparrow); the last species feeds on *P. demoleus* visiting the flowers. Girish Kumar (2017) reported that *C. ternatea* is pollinated by *Amegilla cingulata, Xylocopa latipes, X. aestuans* (bees), *Jamides celeno* (Lycaenid butterfly), *Udaspus folus* (Hesperiid butterfly), Zygaenidae moth, *Spoladea recurvalis* (Crambid moth). In this study, the resupinate flowers of *C. ternatea* is pollinated primarily by bees and supplemented by butterflies. Among bees, *Anthophora* bees sometimes resort to nectar robbery by making punctures at the base of the corolla and/or calyx tube and bypassing the sexual apparatus placed in the corolla tube. This illegitimate nectar collection results in standing crop of nectar without effecting pollination but it indirectly promotes visits by bees that probe the flowers legitimately effecting pollination. A beetle, *Mylabris phalerata* feeds on the corolla and sex organs of flowers and its activity surely affects the success of sexual reproduction in *C. ternatea*.

In this study, *C. ternatea* fruits dehisce to shatter seeds to short distances and the fallen seeds disperse subsequently by rain water during wet season indicating that this plant species is both autochorous and hydrochorous, the former mode facilitates population build up in the parental sites while the latter mode facilitates invasion of new habitats enabling the expansion of its distribution range. Staples (1992) also mentioned that *C. ternatea* throws out seeds vigorously from the dehiscing dry pods and the seeds also dispersed in cattle dung. Girish Kumar (2017) reported that *C. ternatea* displays vivipary to overcome aseasonal rains but such a situation has not been observed in the habitats of *C. ternatea* in the present study.

C. ternatea is widely used in Indian Medicine System (Kirtikar and Basu 1991) and in Ayurvedic medicine (Mukherjee et al. 2008) for treating various human diseases and ailments. The plant is cultivated as forage and fodder crops, a source of natural food colorants and antioxidants, and as an ornamental plant (Reid and Sinclair 1980; Barro and Ribeiro 19983; Hall 1985; Jain et al. 2003;Gomez and Kalamani 2003; Cook et al. 2005; Prafulkumar 2011). This species is often cultivated soil fertility and enhance crop yields in maize, sorghum and wheat (FAO 2016) and as a re-vegetation species for the rehabilitation of coal mines (Cook et al. 2005). Since *C. ternatea* has multiple uses, it can be cultivated in areas where it is not a menace to use it for different purposes as stated by different authors.

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5. CONCLUSIONS

Clitoria ternatea is an aggressive colonizer of several habitats with poor to moderate soil nutrient environment. It has blue- and white-flowered varieties with similar floral structural and functional characters. It produces chasmogamous nectariferous resupinate hermaphroditic flowers with brush pollination mechanism and weak protandry adapted for insect pollination, primary by bees. Autonomous autogamy and facultative xenogamy are functional to maximize fruit and seed set rates in open-pollinations. Seed dispersal modes include autochory and hydrochory; the former enables population build up in parental sites while the latter to occupy new habitats and expand their distribution sites. This plant is widely used in Indian Medicine System for treating various human diseases and ailments, as forage and fodder crop, natural food colorant, antioxidant, ornamental plant and as a re-vegetation species in the coal mine sites. Therefore, *C. ternatea* is commercially and ecologically valuable.

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Authors contributions:

Both authors contributed equally.

Ethical approval

The ethical guidelines for plants & plant materials are followed in the study for species collection & identification.

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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